ACTIVITY IN A CRUSTACEAN GANGLION. I. CARDIO-INHIBITION AND ACCELERATION IN PANULIRUS ARGUS[1]

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The cardiac nerves of arthropods have been studied from time to time (Krijgsman, 1952), but there has been no extended work on the decapod Crustacea. Much has been done on *Limulus* (Carlson, 1905, 1905–06; Heinbecker, 1933, 1936; Garrey, 1942; Prosser, 1943) which seems to have an analogous system with respect to cardiac ganglion and extrinsic nerves, but the ganglion contains a number of cells and by its consequent complexity is of limited usefulness in determining the mechanism of its action as a pacemaker for the heart beat and its relation to the central nervous system.

The decapods have a relatively simple ganglion, or one containing a limited number of cells (Alexandrowicz, 1932) and relatively few extrinsic fibers connecting the ganglion with the central nervous system. The normal heart beat has been shown to have its origin in a burst of nervous activity in the cardiac ganglion (Welsh and Maynard, 1951). A study of some of the details of this activity has been made and will be presented in another paper. Briefly, the frequency of beat is determined by the frequency of the bursts of nervous activity, and the amplitude of contraction is determined by the number of motor impulses per beat. Each cell, as in *Limulus* (Prosser, 1943), may "fire" a number of times per burst.

The heart beat may be augmented or inhibited, both in amplitude and rate, if the proper extrinsic nerves connecting the ganglion with the central nervous system are stimulated. Wiersma and Novitski (1942) give an excellent account of the qualitative effects of the extrinsic nerves in the crayfish heart, and Smith (1947) studied the crab heart and the pharmacology of the extrinsic nerves. However, no quantitative study of the effects of these nerves has been published. The present investigation was undertaken to determine the effects of varying parameters of stimulation of each of the extrinsic nerves. The cardio-inhibitor is of particular interest, for if its action is upon the ganglion, as seems to be the case, this is a preparation in which an inhibition, possibly analogous to central inhibition, may be studied. The pertinent anatomy was worked out, and a general description, supplementing that of Alexandrowicz (1932), is given.

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Materials and Methods

Thirty-eight adult *Panulirus argus* (Latreille), the Bermuda spiny lobster, were used. Antennae and legs of the lobsters were removed, the ventral nerve cord severed at the base of the abdomen to prevent movement, and the animal allowed to bleed. The carapace anterior to the cervical groove was then removed, and the viscera dissected out. Care was taken to prevent leakage of digestive fluids into the cavity, which was washed with sea water upon removal of the organs. The heart was perfused with sea water by means of a cannula inserted into the opening of the ophthalmic artery. After a period of initial irregularity, the heart usually beat regularly and continuously for several hours at room temperature, about 27° C.

For morphological study, the heart or portions of the central nervous system were stained with dilute methylene blue in sea water. The preparation was usually studied fresh, and then fixed in ammonium molybdate (Alexandrowicz, 1932).

In such a preparation, the cardio-inhibitors are exposed on the surface of the abdominal flexors; the first and second cardio-accelerators may be exposed by cutting and lifting the first and second abdominal flexors. The accelerators are also visible for a short distance laterally above the muscles before entering the pericardium.

Silver wire hook electrodes 2.5 mm. apart were used for stimulation. Square stimulus pulses were supplied by a Grass 3-BC stimulator.

The heart contraction was recorded by kymograph. The recording lever was attached to the cannula perfusing the heart rather than to the heart directly.

The percentage of acceleration or inhibition was obtained by dividing the change in beat by the normal rate determined immediately before stimulation. The heart rate was not constant, usually varying plus or minus 10% during a run of several minutes and some 20% over several hours. The average maximum and minimum rates recorded in the normal beat of 11 animals were 71.2 and 47.8 beats per minute. The absolute maximal and minimal rates for these animals were 115 and 29.5 beats per minute. In general, a difference of 10% is significant in determining the effects of the extrinsic nerves.

Results

Anatomy

The cardiac ganglion (Alexandrowicz, 1932; Welsh, 1939) is similar to those of other marine decapod Crustacea. It is Y-shaped and lies on the inside of the dorsal wall of the heart, crossed by one or two muscle strands. There are five large ganglion cells; four of these are grouped anteriorly, close together at the fork of the "Y", the fifth occurs about half-way down the stem at the junction of prominent side branches. In the posterior half of the stem, there are four smaller cell bodies arranged linearly with more or less equal spacing. The large cell bodies averaged $54 \times 84\,\mu$ in fixed preparations, and the small cells, $22 \times 43\,\mu$. Fibers from the ganglion cells first run in the central trunk of the ganglion and then branch into the myocardium, ramifying throughout the muscle. They also give off collaterals which form neuropiles within the trunk or extend into the muscle to form arborizations (see Alexandrowicz, 1932).

The cardiac ganglion is connected to the central nervous system by fibers which arise in the thoracic ganglion and reach the heart via the inhibitor or accelerator nerves, the pericardial plexus, and the dorsal nerve (Fig. 1A).

The pair of cardio-inhibitor nerves (CI) arises in the anterior region of the thoracic ganglion. On each side, one of the pair runs forward along the sternal canal to the cephalic apodeme, where it leaves the canal, passes through a small opening or notch in the endophragmal plate, and then runs dorsally along the surface of the abdominal flexors to the anterior border of the pericardium. There it enters the pericardium and joins the lateral pericardial plexus (pp).

The two pairs of cardio-accelerators (CA-1 and CA-2) arise immediately posterior to the cardio-inhibitor in the ganglion. The second is the most anterior of a pair of nerves arising from the base of the nerve to the first pereiopod; the first accelerator is anterior to this, presumably from the region at the base of the third maxilliped nerve. These would correspond to segmental nerves "m" and "I" which were described by Heath (1941) in the crab, *Pugettia*. They run forward along the sternal canal for a short way, but leave it through sinuses in the endophragmal plate in the first and second segmental region, respectively, behind the cardio-inhibitor. Then they run dorsally beneath the abdominal flexors to the pericardium where they enter the lateral pericardial plexus. The posterior cardio-accelerator branches before entering the pericardium, its anterior branch going to the plexus while the posterior branch proceeds to the pericardium sending off twigs to the muscles of the dorsal region and to the ligaments of the heart.

A pair of dorsal nerves (DN) carries the accelerator and inhibitor fibers from the lateral pericardial plexus to the heart. On leaving the plexus, one of the pair may contain as many as 11 methylene blue-stainable fibers; one of these branches off soon after leaving the plexus and enters the dorsal musculature. The others remain until the nerve reaches the lateral suspensory ligaments where the nerve passes through another plexus which ramifies over the ligaments. Here most of the fibers in the dorsal nerve branch and leave until two to four are left which actually enter the heart. These fibers can be traced back through the ligamental plexus (lp) and shown to proceed directly along the nerve from the lateral plexus. They may be considered as cardio-regulators, while the additional number originally in the dorsal nerve appear to be concerned with some function in the ligaments. As shown in Figure 1A, the ligamental plexus is composed of ramifications of branches from the lateral plexus. The posterior ligamental plexus may also receive branches from the posterior branch of the second cardio-accelerator.

The dorsal nerve enters the heart dorso-laterally between the dorsal ostia (Fig. 1A). After a slight swelling at the surface of the myocardium, it usually runs directly to the ganglionic trunk at the level of the third or fourth large cell. There is much variation; the path of the nerve on each side of the heart is usually different, and in some cases small branches are given off to the muscle or there is an anastomosis with nerve branches from the central trunk. It could not be ascertained whether the small branches contain collaterals from the regulator fibers. In the anastomoses, there was no union of the actual nerve fibers; the sheath of connective tissue is merely united.

Alexandrowicz (1932) distinguished thick and thin fibers within the dorsal nerve which he termed System I and System II. In the dorsal nerve of *Panulirus*, a maximum of four fibers, similar in size (5 to 9μ), stained well with methylene

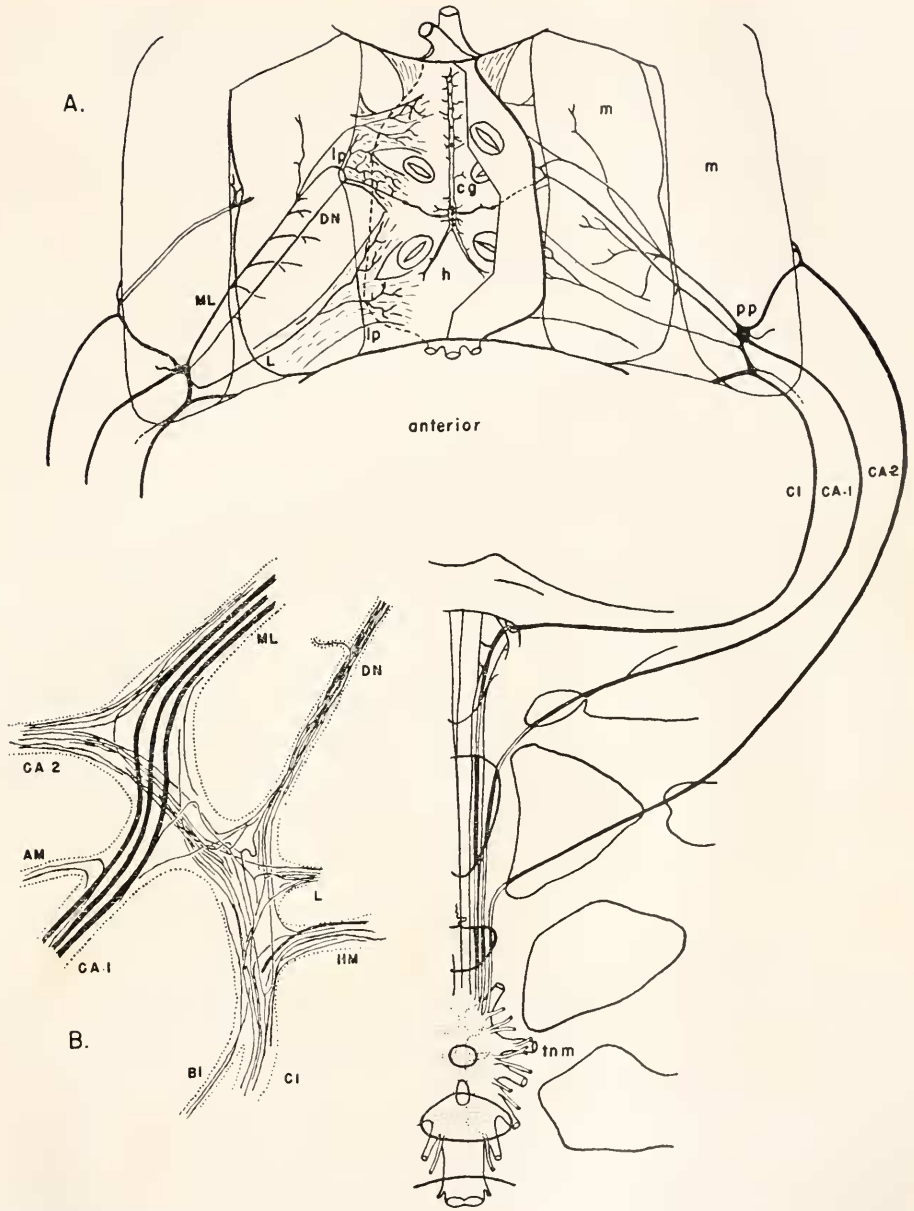

FIGURE 1. Cardiac nerves of *Panulirus argus*. A. Thorax split horizontally and upper-half laid back, giving dorsal view of thoracic ganglion and ventral view of heart. The heart is slightly enlarged with ½ ventral side removed to show cardiac ganglion. B. Left lateral pericardial plexus, enlarged to show nerve fibers. AM, branch to dorsal abdominal muscles; BI, branch from cardio-inhibitor; CA-1, first cardio-accelerator; CA-2, second cardio-accelerator; cg, cardiac ganglion; CI, cardio-inhibitor; DN, dorsal nerve; h, heart; HM, nerve to hypodermis and dorsal muscles; L, ligamental nerve; lp, ligamental plexus; m, muscles; ML, nerve to ligaments and dorsal muscles; pp, lateral pericardial plexus; tnm, thoracic ganglion.

blue. These correspond to System I. Upon entering the ganglion they divide, sending equal processes anteriorly and posteriorly in the trunk. These immediately send out small branches which either form networks over the surface of the large cells or help form neuropiles within the ganglion. The main processes can be traced along the trunk and seen to send out occasional branches. According to Alexandrowicz, such branches end in the muscle close to the endings of the ganglion cell fibers. The regulator fibers would therefore act both in the ganglion on cell bodies and in the muscle.

System II stained only in rare instances when smaller fibers were noted entering the ganglion with System I. They may have also been present but unidentifiable in the general fiber complex of the trunk.

Figure 1B gives semi-diagrammatically the general relationships of the nerve fibers entering and leaving the lateral plexus. There are about nine nerves involved: 1) the cardio-inhibitor, 2) a branch at the base of the cardio-inhibitor which proceeds along it for a distance (BI), 3) the first cardio-accelerator, 4) a branch containing two fibers from the first cardio-accelerator which runs to the abdominal extensor (AM), 5) the second cardio-accelerator, 6) a branch which contains large motor fibers from the first cardio-accelerator and smaller fibers supplying the suspensory ligaments of the heart (ML), 7) the dorsal nerve, 8) the anterior ligamental nerve, running to the anterior suspensory ligaments of the heart, 9) a branch consisting largely of fibers from the cardio-inhibitor which do not cross the plexus but appear to run dorsally into the muscles and hypodermis (HM). The nerve paths may vary greatly, especially (6), which as 'ml' in Figure 1 follows a different course on each side. The number of fibers shown in Figure 1B is not complete, for all did not stain with methylene blue. A number of fibers entering the dorsal nerve branch before entering, while there are some from each of the cardio-accelerators and the cardio-inhibitor which do not branch. It is among these latter that the actual regulator fibers are probably found, while the branching fibers are presumably those which innervate the ligaments of the heart.

Physiology

1. *Cardio-inhibitor*

Both the number of active fibers and the frequency or pattern of impulses per fiber presumably act in producing a graded effect on the heart beat. In analysis, therefore, the response of the nerve to stimulation was separated from the response of the heart to the inhibitor nerve.

a. Excitability of inhibitory fibers. A frequency was chosen (70 per second) which had been shown to give complete or nearly complete inhibition. This was kept constant and the threshold at varying strengths and pulse durations determined (Fig. 2). The pseudochronaxie was 0.2–0.5 milliseconds, and k value (Hill's time constant), 0.29–0.72. In one case, not in Figure 2, a pseudochronaxie of 1.45 milliseconds was obtained.

The absolute values of the pseudochronaxie, though subject to errors due to polarization of the electrodes, are of the same order of magnitude as others obtained for crustacean nerves (Jasper and Monnier, 1933). The lack of irregularities in the curve indicates that a single physiological unit is being stimulated. At threshhold

voltage, the response was often merely a temporary hesitancy in the heart beat, indicating a transitory stimulation at the electrodes.

If either voltage or pulse duration was well above minimum threshold value, increasing the other parameter caused a stepwise, not graded, increase in inhibition. Thus, an incompletely inhibited heart showed no effect as the pulse duration was increased several hundred per cent. Then suddenly a second threshold level was reached and the heart became completely inhibited. Usually there were two such levels, though in one case there were three, and in several instances there was no increase over the first minimum threshold effect. If either parameter was near minimum threshold value, the second level could not be obtained (Fig. 4). It thus has the qualities of a true second threshold.

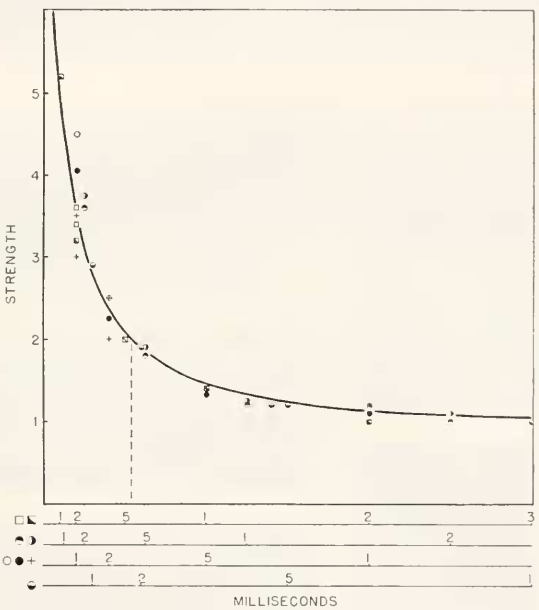

FIGURE 2. Strength-duration curve. Ordinate in multiples of rheobase. ○, cardio-inhibitor; □, first cardio-accelerator; +, second cardio-accelerator.

With increasing voltage and a constant pulse duration, there was a small but definite range (0.05–0.2 volts) at minimum threshold and at each step where there was a gradation before the final level of inhibition was reached. It would appear that in this relatively narrow range the fiber responds to some but not all of the stimuli, but that after the fiber responds to each stimulus, a change in voltage no longer causes increased response in that fiber.

In several instances it was apparent that the fibers running to either the ligamental plexuses or the dorsal musculature were stimulated, for a change in the base line not otherwise associated with the heart beat was obtained upon stimulation.

With possible exceptions at low frequencies of stimulation, repetitive responses to each stimulus did not occur. The regular relation between frequency and effect,

the fact that changing pulse duration and voltage did not result in a consequent gradation in effect, and the low pseudochronaxies argue for this conclusion.

Exhaustion of the cardio-inhibitor was noted in cases of long-continued stimulation. There was either a gradual and irregular, or sudden increase in the heart

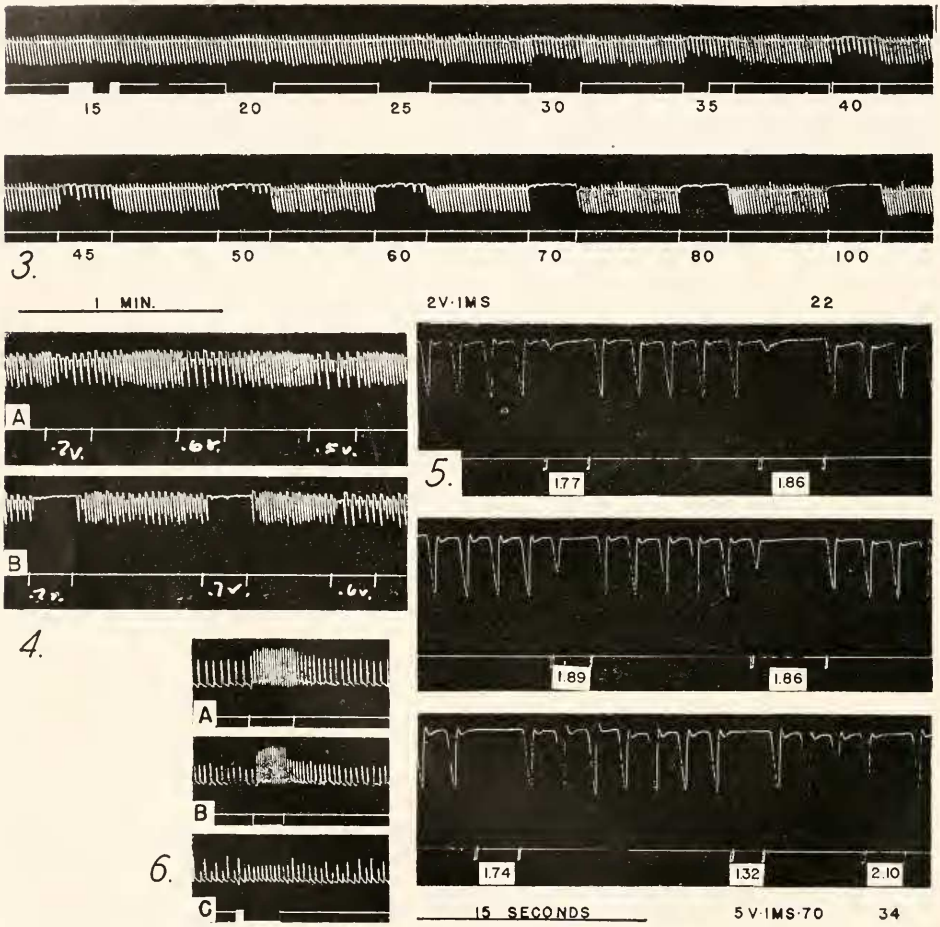

FIGURE 3. Cardio-inhibitor. Frequency varied; stimulus strength, 2 volts, 1 millisecond pulse duration.

FIGURE 4. Cardio-inhibitor; interrelation of pulse duration and stimulus strength. Stimulus frequency, 40 per second, voltage varied. A. Pulse duration, 0.2 milliseconds; B. pulse duration, 0.5 milliseconds. Note alternating beat.

FIGURE 5. Cardio-inhibitor. There is progressively less effect on the beat immediately following the initiation of stimulation as the time between these events is decreased. Stimulation at 70 per second, 5 volts, 1 millisecond.

FIGURE 6. Cardio-accelerator. A. Cardio-accelerator 1; stimulation at 50 per second, 1.4 volts. B. Cardio-accelerator 2; stimulation at 50 per second, 0.5 volts. Note the after-effect. C. Cardio-accelerator 2; stimulation at 15 per second, 5 volts. The alternating beat is abolished upon stimulation, but returns upon the release of stimulus.

beat. Since it was found that the recovery process following accomodation or exhaustion of the cardio-inhibitor was slower or followed a different time course from the accomodation itself (Levin, 1927), a 15-second stimulus period was usually followed by a 30-second rest in determining the stimulus-response curve. This in most cases proved adequate to maintain the nerve in an unexhausted state.

b. Response of the heart to the inhibitor. At a given level the percentage of inhibition obtained was characteristically a non-linear function of the frequency of stimulation. In most cases complete inhibition was obtained by stimuli at 100 per second. Exhaustion of the nerve was frequent at frequencies between 80 and 500, and the threshold of stimulation was often raised (Pantin, 1936). The upper limit of the frequency of stimulation appears, when obtained, to be due to the failure of the cardio-inhibitor rather than to some effect at the cardiac ganglion. The minimum frequency producing detectable inhibition was about 10–15 per second (Fig. 3). In several instances stimulation at 15 per second or less had slightly more effect than stimulation at 20 per second.

As the frequency of stimulation was increased, the percentage of inhibition, as measured by decreased frequency or amplitude of beat, increased. Because each of the two or three threshold levels gave a different stimulus-response curve, it was impossible to average the results of all experiments. Values obtained for six animals were grouped roughly according to the pulse duration of the applied stimulus (Fig. 7). Curve 1 is above the first threshold, the pulse duration is rather short. In Curve 2, the second threshold level, the pulses were of long duration, and in Curve 3 are the long pulses of one preparation which also showed threshold levels 1 and 2. With aging the thresholds tended to change, and in several instances the older preparations showed only the first threshold effect.

Usually the inhibited amplitude and frequency remained regular and in some instances served to regularize a normally irregular beat. Occasionally, however, stimulation of the inhibitor caused an irregular, inhibited beat. Since this irregularity was also noted near the nerve threshold, it seems most likely that it was because of the irregularity of the impulses arriving at the ganglion. In certain other instances a beat alternating between a strong and weak contraction (Fig. 4) was initiated. This seems to be a peculiarity of the ganglionic action and was very often noted in old or slightly injured preparations. It is also found in non-nervous but rhythmically active centers such as the molluscan heart (Welsh and Taub, 1950, Fig. 1). Near the frequencies causing complete inhibition it was not unusual to find the heart contractions either quite arrhythmical or of varying strengths. It is not clear whether these, and the occasional escape beats which occurred during otherwise complete inhibition, are due to changes in the threshold of the cardio-inhibitor nerves and consequently the number of impulses arriving at the ganglion, or whether they are due to some change in the threshold of the ganglion's response to the arriving impulses.

Facilitation is present, but the period of facilitation must be quite short. At frequencies of 20 per second, there is some evidence that there is a progressive inhibition during the first few beats. At higher frequencies the heart beat is much too slow to indicate facilitation in the ganglion, and inhibition is immediate. As shown below, a stimulus of 70 per second may produce its maximum effect in less than $\frac{1}{3}$ of a second.

If, in a heart beating approximately every two seconds (Fig. 5), a stimulus of 70 per second was applied up to 1.7 seconds after a beat, the next beat was completely inhibited. If, however, the stimulus was applied between 1.7 and 2.1 seconds after a beat, the following heart contraction was only partially inhibited. Thus for a period of ⅓ second, a progressive inhibition in the beat following the initiation of the stimulus was obtained. It would seem most probable that this is accomplished by blocking a portion of the nervous activity in the ganglion, and that the ⅓ second measures the maximum duration of facilitation at this frequency of stimulation.

FIGURE 7. Cardio-inhibitor; stimulus-response curves for 6 animals. Curve 1, pulse duration, 0.2 to 5 milliseconds; Curve 2, 1 to 10 milliseconds; Curve 3, 5 to 10 milliseconds. In any one animal there was a sharp division between curves, but the thresholds varied among animals.

Four phenomena may be noted upon cessation of inhibitory stimulation: 1) there may be a series of beats of gradually increasing frequency and amplitude until the normal state is reached; 2) there may be an exceptionally large contraction immediately following the release of inhibition (this may be followed by a period of contractions alternating in amplitude and frequency, or a gradual increase as in (1)); 3) there may be a period of accelerated beat and larger amplitude immediately following inhibition; 4) there may occasionally be no noticeable after-effect. The duration of inhibition does not greatly influence the after-effect.

2. Cardio-accelerators

The investigation of the cardio-accelerators was not as extensive as that of the cardio-inhibitor. The pseudochronaxies of both cardio-accelerator nerves (Fig. 2) were not significantly different from those obtained for the cardio-inhibitor. A second threshold was found, though not as commonly as with the inhibitor. Comparisons of the thresholds at different frequencies were not made.

From the responses in Figure 6 and the similarity of the shape of the frequency-acceleration curves, it seems fairly clear that the two cardio-accelerators act in the same manner on the heart, though it is not possible to say that they have the same quantitative effect. In Figure 9A twice as many fibers were probably stimulated in the first cardio-accelerator as in the second. A later series of stimuli applied to

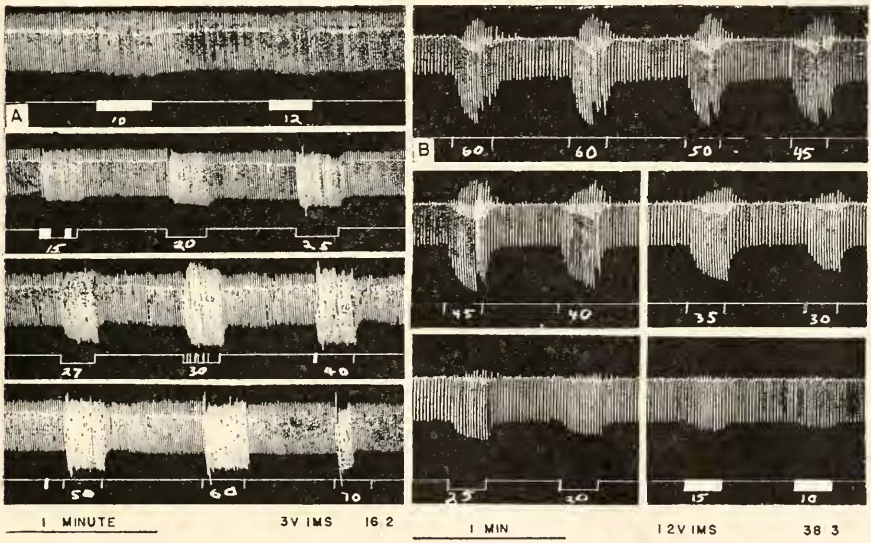

FIGURE 8. Cardio-accelerator; frequency varied. A. Cardio-accelerator 1; stimulation at 3 volts, 1 millisecond. B. Cardio-accelerator 2; stimulation at 1.2 volts, 1 millisecond.

the first cardio-accelerator produced a curve of acceleration with a maximum reduced to approximately that of the second accelerator.

In several instances stimulation of the cardio-accelerators restored a regular beat to an irregular or inactive heart. In such cases, the maximum rate obtained (95–125 per minute) was comparable to the maximum rate obtained from a normally beating heart upon stimulation (74–140 per minute). As Wiersma and Novitski (1942) found, however, the maximum was in many cases correlated with the normal heart rate. The cardio-accelerators usually destroyed an alternating rhythm by equalizing succeeding beats, redistributing a given number of impulses into equal bursts rather than by only increasing the number of impulses in the weaker bursts (Fig. 6).

The effects of varying frequencies of stimulation are shown in Figures 8 and 9.

The amplitude and rate of beat increased in a similar fashion, and there is a maximum effect which is usually reached by stimulation frequencies of 60 per second. No further effect may be obtained with frequencies up to 500 per second. The lower limit of effective frequency varied in the vicinity of 2–5 per second. In a manner similar to stimulation of the cardio-inhibitor, stimuli at 10 per second often

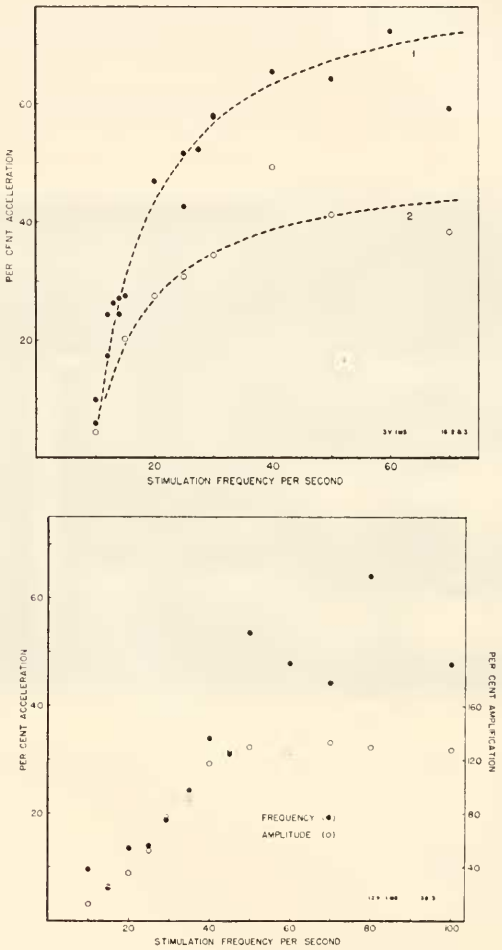

FIGURE 9. Cardio-accelerator; stimulus-response curves. A. ●, cardio-accelerator 1; ○, cardio-accelerator 2. Plot from individual of Figure 8A. B. Cardio-accelerator 2; the response curves are similar in shape for both frequency and amplitude. Plot of individual of Figure 8B.

were more effective than stimuli at 15 per second. It is not clear whether these responses at the lower frequencies involved repetitive discharges in the nerve.

The stimulus-response curves were usually hyperbolic (Fig. 9A), but in several instances a roughly sigmoid curve (Fig. 9B) was obtained. It was found im-

possible to obtain averages of several runs, for the curves varied with time and the individual.

Facilitation of the accelerating mechanism was much slower than of the inhibitor mechanism. It involved a gradual increase in both frequency and amplitude, though the latter effect was usually the more apparent. The slope of this curve, and consequently the duration of facilitation at the maximum response, was related to the frequency of stimulation. In some preparations it was not unusual to find that at lower frequencies (40 per second) facilitation was too rapid to be noted by the amplitude of the heart beat (Fig. 8). In fact, often the first few beats af a period of stimulation were of super-amplitude, caused apparently by the sudden high degree of excitation applied to the pacemaker which destroyed momentarily the equilibrium presumed necessary for a rhythmic beat. This could also be considered a period of facilitation if it is assumed that a slightly higher level of excitation is necessary to restore regularity at increased rate and amplitude. In some cases where the frequency of stimulation was fairly low (25 per second) there was a slow linear increase in amplitude after the first period of rapid facilitation.

The effects following the release of stimulation can be grouped into three classes. 1) There was an exponential decrease in rate and amplitude, bginning immediately or soon after the release of stimulation. Usually the duration of this was not over 10 seconds, though the final amplitude after stimulation may remain slightly greater than that before stimulation. 2) In three cases the exponential decrease fell to a level of subnormal amplitude and frequency which was followed by a gradual return to the normal beat. In the case of amplitude, this may last for nearly a minute. This effect was especially noted at high frequencies, when low frequencies yielded effect 1, and was to some extent dependent upon the duration of stimulation. 3) In two cases the stoppage of stimulation had very slight or an increased effect. The latter case must have been caused by after-discharge in the nerve. In the former, there was a sudden dip in amplitude upon the release of the stimulus followed by a slow return to normal amplitude lasting well over a minute. The rate returned to normal more rapidly. This is similar to lasting after-effects noted by Smith (1947) in the crab.

Discussion

The System I fibers in the dorsal nerve are probably inhibitory. Their number corresponds well with the number of thresholds, and they make intimate connection with the large cell bodies and neuropiles within the ganglion. The homologous fiber in the stomatopods is inhibitory (Alexandrowicz, 1934). No evidence was obtained concerning the function of System II.

The several thresholds are probably best explained by assuming that new cardioregulator fibers were recruited with increasing stimulus strength. The stepwise increase in inhibition, as mentioned above, can thus be correlated with the limited number of fibers observed (2–4). Likewise the all-or-none type of response at each threshold is as expected if a limited number of fibers of different thresholds were stimulated with a continuously increasing stimulus strength.

Assuming the different thresholds indicate new fibers stimulated, both temporal and spatial summation are clearly shown, especially in the inhibitor. There, temporal summation is slightly more efficient than spatial. Both, however, follow essentially the same "exponential" stimulus-response curve.

Though the stimulus-response curves are quite different from a mathematical viewpoint, they probably do not indicate radically different physiological processes. As shown by Rosenblueth and Rioch (1933) in discussing the response curves of mammalian neuro-effector junctions, the change from an apparent hyperbola to a sigmoid need not indicate a change in the process. Rather, the relative rate of recovery of a preparation following a single nerve impulse, or, as they assumed the rate of destruction of a mediator, in relation to the frequency of stimulation could determine whether a sigmoid or a hyperbola would be obtained. Thus the sigmoid frequency-acceleration curve would indicate that the recovery following each impulse was more rapid in this preparation than in one which produced a hyperbolic curve. The inhibitor stimulus-response curve could also fit here, in which case it would correspond to the lower portion of a sigmoid. Complete inhibition would generally be reached before the flexion point. The short after-effect of inhibition as compared with acceleration is evidence for the required rapid recovery.

These curves, however, do not indicate whether the process is via the release of a chemical mediator as Rosenblueth and Rioch felt. It seems possible that an electrotonic potential, or some other effect, built up by a series of pulses could be the inhibiting or accelerating stimulus.

In any case, if an inhibiting mediator is present, it is probably not that noted by Parrot (1941) in the blood stream after inhibitory stimulation, which caused activity in the intestine, for this was obviously not rapidly destroyed.

The alternating rhythm deserves some mention. In the normal heart beat, a burst of nervous activity is followed by a silent period and then another burst (unpublished observation). The continuity of this regular action is assumed to depend on a balance between activity and recovery of all the cells of the ganglion. Under some conditions the excitability states of the ganglion cells get out of phase. A burst of activity may be just great enough to prevent all the cells from recovering completely by the time the next burst is initiated by the most rapidly recovering cell. Therefore, the second burst is smaller, some of the cells "fire" fewer times than before, and the following recovery period is shorter, for the interval seems dependent upon the activity during the burst. In the next burst, the number of cells completely recovered is greater. Therefore, there is a large burst that is followed by a smaller, and so on. If the excitability of the out-of-phase cells can be either raised or lowered, a regular beat should be re-established. As a corollary, if the excitabilities of some of the cells of a regularly beating heart are either raised or lowered more than of other cells, throwing them out of phase, the alternating beat should be established. Both of the above phenomena were observed upon stimulation of the extrinsic nerves. Usually the accelerators stopped the alternating beat, though in some instances a temporary alternation was begun in an otherwise regular beat. The inhibitors generally brought about the alternation, though at times they also served to stabilize. If the above is correct, we may, therefore, conclude that the extrinsic nerves appear to affect the excitabilities of the individual ganglion cells.

I wish to acknowledge the aid of Dr. John H. Welsh in formulating this problem and the cooperation of Dr. L. Hutchins and Dr. W. Sutcliffe at the Bermuda Station. I also wish to express appreciation to Dr. R. I. Smith and Dr. T. H. Bullock for criticizing the manuscript.

Summary

The morphology and aspects of the physiology of the cardiac ganglion and the extrinsic cardiac nerves of *Panulirus argus* have been studied.

1. The extrinsic nerves consist of two pairs of cardio-accelerators and one pair of cardio-inhibitors which arise in the anterior portion of the thoracic ganglion. These run dorsally and laterally to join at the pericardial plexus where fibers from each come together to form the dorsal nerve. This runs to the cardiac ganglion. The thick fibers (2–4) of the dorsal nerve which ramify over the large cells of the ganglion are probably inhibitors. The identification of the accelerator fibers remains unclear.

2. Upon electrical stimulation of the extrinsic nerves, two or three levels of response were obtained with increasing pulse duration and voltage. This was probably because of an increase in the number of fibers stimulated.

3. Complete inhibition was usually obtained by frequencies of 35, 50 or 90 stimuli per second, depending upon the level of response of the heart. Usually no inhibition was obtained below a frequency of 15 per second. Between the minimum and maximum there was a gradation of both frequency and amplitude of heart beat. The stimulus-response curves approximated an exponential curve. Facilitation and an after-effect were present but of short duration.

4. The maximum increase in frequency and amplitude of the heart beat was usually obtained by stimulation of the accelerators at 60 per second or more. No response was ordinarily obtained below 5–10 stimuli per second. The stimulus-response curve was usually hyperbolic, though in some cases it was sigmoid. The actions of the two accelerators appear to be identical. Facilitation and after-effect have a much longer time constant than in the case of the inhibitor.

5. From a consideration of the participation of individual cells in the cardiac ganglion discharge for each beat, it is probable that the cardio-inhibitor and accelerators depress or enhance the excitabilities of the ganglion cells.

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